Chapter 9

Myocardial Connexin-43 is Implicated in the Prevention of Malignant Arrhythmia in Rats Suffering from Essential Hypertension

Tamara Egan Benova, Barbara Szeiffova Bacova, Csilla Viczenczova, Miroslav Barancik and Narcis Tribulova

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Abstract

Gap-junction connexin (Cx) channels are important determinants of myocardial conduction and synchronization that is crucial for heart function. Hypertension-induced structural remodeling is associated with an increased risk of life-threatening arrhythmias and heart failure in both humans and experimental animals. Recent studies suggest that abnormal distribution and/or downregulation of Cx43 accompanied with altered protein kinase C (PKC)ε signaling in spontaneously hypertensive rats were linked with increased propensity to ventricular fibrillation compared to normotensive rats. By contrast, the long-term treatment of hypertensive rats with cardioprotective compounds such as melatonin, omega-3 fatty acids, or red palm oil resulted in protection from lethal arrhythmia. Their antiarrhythmic effect was attributed to the attenuation of abnormal Cx43 topology and modulation of Cx43 mRNA as well as protein expression and its functional phosphorylated forms. The latter might be attributed to upregulation of PKCε. It appears that maladaptive consequences of hypertension resulting in abnormal myocardial distribution of Cx43 and its downregulation can contribute to arrhythmogenesis and occurrence of malignant arrhythmias. On the other hand, the attenuation of myocardial Cx43 abnormalities by treatment with melatonin, omega-3 fatty acids, or red palm oil confers arrhythmia protection in rodent model of essential hypertension. Findings uncover novel mechanisms of cardioprotective effects of melatonin, omega-3 fatty acids, and red palm oil. Well-designed clinical trials are needed to explore antiarrhythmic potential of these compounds in human essential hypertension.

Keywords: hypertension, arrhythmias, connexin-43, melatonin, omega-3 fatty acids

1. Introduction

Different types of epidemiologic studies, including a growing number of large cohort studies and interventional trials in different population groups, have consistently shown that there is a strong positive association between hypertension and cardiovascular diseases (CVDs), and that lowering the blood pressure helps to improve cardiovascular health. Hypertension is the leading risk factor for the development of CVD, with men exhibiting a higher arterial pressure than women during the ages from 20 to 65. Up until menopause, there are significant sex differences in blood pressure, vascular reactivity, and renal function [1].

Hypertension is a multifactorial process. Environmental factors and the genetic component as well as epigenetic inheritance contribute significantly to essential hypertension [2] that is known to be on rise in the human population. Sympathetic nervous system plays a major role in the maintenance of hypertension and the rostral ventrolateral medulla is the main source of this sympathetic activation. It is proposed that melatonin-induced epigenetic modifications in the neurons of area postrema are implicated in this process [3]. Sympathetic activation in hypertension is accompanied by oxidative stress and inflammation as by-product [4]. These undesired processes might be ameliorated by interventions with compounds possessing antioxidant and free radical-scavenging ability, for example, melatonin, statins, adrenergic beta receptor blockers, and omega-3 fatty acids. Patients with uncontrolled essential hypertension have elevated concentrations of superoxide anion, hydrogen peroxide, lipid peroxides, endothelin, and transforming growth factor-beta with a simultaneous decrease in endothelial nitric oxide, superoxide dismutase, vitamin E, and long-chain polyunsaturated fatty acids (PUFAs) [5–7]. The implication of redox signaling and lower omega-3 index is suggested in the pathogenesis of essential hypertension in animal models as well [8, 9]. Omega-3 fatty acids exhibit wide-ranging biological actions [6] that include the regulation of renal sodium excretion and vasomotor tone, partly by decreasing the production of vasocostrincting and anti-inflammatory eicosanoids. Omega-3 fatty acids also activate the parasympathetic nervous system. It is proposed that the availability of adequate amount of omega-3 fatty acids during the critical periods of growth prevents the development of hypertension in adulthood [10].

Hypertension is a major risk factor for cardiovascular injury resulting in heart attack, congestive heart failure, stroke, as well as sudden arrhythmic death [11, 12]. The latter is associated with myocardial structural remodeling that follows hypertension, such as hypertrophy and fibrosis. This remodeling is accompanied by changes in expression, distribution, and function of cell membrane ion channels, intercellular gap-junction connexin-43 (Cx43) channels, Ca^{2+}-cycling proteins, and extracellular matrix composition [13–16]. Mentioned remodeling

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predisposes to arrhythmogenic mechanisms including early or delayed after-depolarization and reentry of excitation, facilitating life-threatening ventricular tachycardia (VT) and ventricular fibrillation (VF).

2. Hypertension-related gap junctions and connexin-43 remodeling

Changes in cardiac workload due to pressure or volume overload induce hypertrophic growth of individual myocytes. Hypertrophy of cardiomyocytes counteracts the increased wall tension (Laplace's law), and is therefore often considered as compensated hypertrophy [17]. However, prolonged state of hypertrophy is accompanied by maladaptation that promotes progression into heart failure (decompensated hypertrophy). Typical ultrastructural alterations of cardiomyocytes from the left ventricle of old spontaneously hypertensive rats (frequently used model mimicking essential hypertension in humans) are demonstrated in

Figure 1. Representative electron microscopic images demonstrating cardiomyocytes and intercellular junctions in the left ventricles of spontaneously hypertensive rat hearts. (**A**) Conventional ultrastructure showing electron-dense mitochondria (m), adhesive fascia adherens (FA) junctions at the intercalated disk, and peripheral gap junction (arrows) on the lateral sides of the cardiomyocytes. (**B**) Development of lateral gap junctions following the formation of adhesive junctions (AJs) and high amounts of ribosomes (R) and dense mitochondria (m) are seen in young hypertensive rat heart at compensate stage of hypertrophy. (**C**) Severely injured cardiomyocytes exhibiting edematous mitochondria, myocytolysis, pronounced reduction of adhesive fascia adherens (FA) junctions, and loss of gap junctions are sporadically seen in the myocardium of old hypertensive rats at early decompensate stage of hypertrophy.

Figure 1. The cardiac remodeling process is characterized by both structural and electrical disorders that decrease the electrical stability of the heart [16, 18]. A hallmark of the electrical changes with regard to impulse conduction is an impairment of electrical coupling due to abnormal expression of Cx43-constituted gap junctions. Available data suggest that particularly spatial heterogeneity and severity of Cx43 channels dysfunction throughout myocardium affects myocardial conduction and electrical properties of the heart. In addition to structural remodeling, hypertension likewise other systemic or heart disease and proarrhythmogenic conditions are linked with oxidative stress and/or inflammation [4, 7, 9]. This pathology contributes to the impairment of intercellular junctions and communication due to the acceleration of Cx43 degradation and/or dysfunction as well as other Cx43-interacting proteins [19]. Cascade events induced by hypertension resulting in an increased risk for malignant arrhythmias are demonstrated in **Figure 2**.

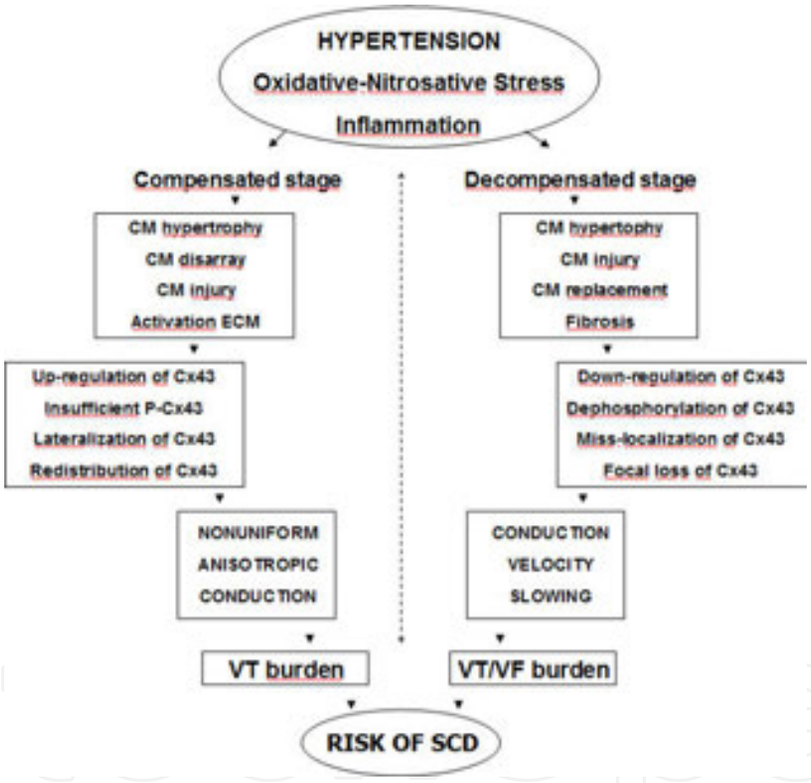

Figure 2. Scheme showing possible myocardial alterations of cardiomyocytes as well as Cx43 expression and distribution that might increase a risk for SCD in spontaneously hypertensive rats and also most likely in patients suffering from essential hypertension.

Several studies have described changes in the number, size, and distribution of myocardial gap junctions during hypertrophic heart disease. In general, Cx43 expression appears to be unaltered or upregulated during the initial and compensatory phase of hypertrophy but they are always redistributed along the cardiomyocyte surface (**Figures 3** and **4**). Cx43 expression and the number of intercalated disk-related gap junctions are reduced when the hypertrophy becomes maladaptive, accompanied by severe cardiomyocyte injury (**Figures 3** and **4**) and

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interstitial fibrosis resulting in progression to heart failure [16]. Kostin et al. [15] reported that in the left ventricles of pressure-overloaded human hearts with aortic stenosis, Cx43 expression was increased in the compensated hypertrophic stage, but was decreased and heterogeneously distributed throughout the ventricles in the period of decompensated hypertensive heart. The decreased expression of Cx43 at the protein level is accompanied by a reduction of Cx43 mRNA, suggesting that the downregulation of Cx43 in hypertrophic heart disease is regulated at the transcriptional level. Downregulation and/or heterogeneous redistribution of Cx43 channels is often associated with abnormal conduction that facilitates arrhythmias (**Figure 2**), although there seems to be a large reserve before reduced intercellular coupling becomes arrhythmogenic [18]. Interestingly, hypertrophic cardiomyopathy, the most common genetic disease of the myocardium, is also characterized by cardiomyocyte hypertrophy, myofibrillar disarray, fibrosis, and miss-localization of Cx43 linked with changes in myocardial conduction and prolonged QRS interval [20]. Consequently, there is a high risk of sudden arrhythmic death, especially in young adults (including competitive athletes).

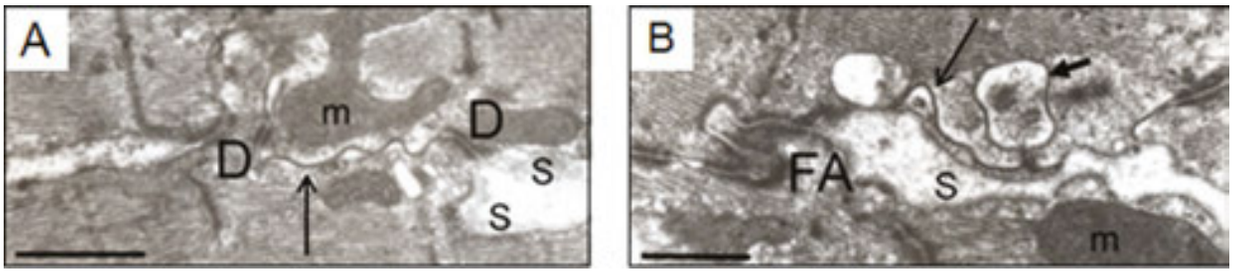

Figure 3. Representative electron microscopic images demonstrating altered topology of myocardial gap junctions in hypertensive rat hearts. (**A**) Lateraly situated gap junctions in the vicinity of adhesive junctions are seen in young rats frequently at compensated stage of hypertrophy. (**B**) Internalization of intercalated disk-related gap junctions (arrows) is often seen in old rats at decompensated stage of hypertrophy. Abbreviations: s – sarcolemma, D – desmosome, FA – fascia adherens junction, m – mitochondria, bar 1 μm.

In the context of myocardial remodeling and Cx43 alterations, it is important to note that microRNA-1 has an essential regulatory impact in cardiogenesis, cardiac hypertrophy, and cardiac electrophysiology. The latter is due to its ability to modulate the expression levels of molecular targets that modulate the electrical properties of cardiac cells. These targets are GJA1-encoding Cx43 and KCNJ2-encoding potassium channel protein that determine myocardial conduction velocity and repolarization [21]. Downregulation of microRNA-1 at the early stage of cardiac hypertrophy was associated with an increased Cx43 protein levels and enhanced Cx43 phosphorylation. The latter correlated with the displacement of Cx43 from the gap junctions that facilitated ventricular tachyarrhythmias [22]. In turn, it is most likely that decompensated hypertrophic stage accompanied by a decrease of Cx43 gene transcripts and protein levels might result from upregulation of microRNA-1. This view is supported by findings that inflammation (known to be implicated in the pathogenesis of hypertension) represses Cx43 expression via upregulation of microRNA-1 and potentiates arrhythmogenesis by targeting GJA1 [23, 24].

Taken together, it can be hypothesized that the prevention or attenuation of maladaptive myocardial Cx43 remodeling and dysfunction induced by hypertension could decrease a risk of arrhythmic death and heart failure [18, 25].

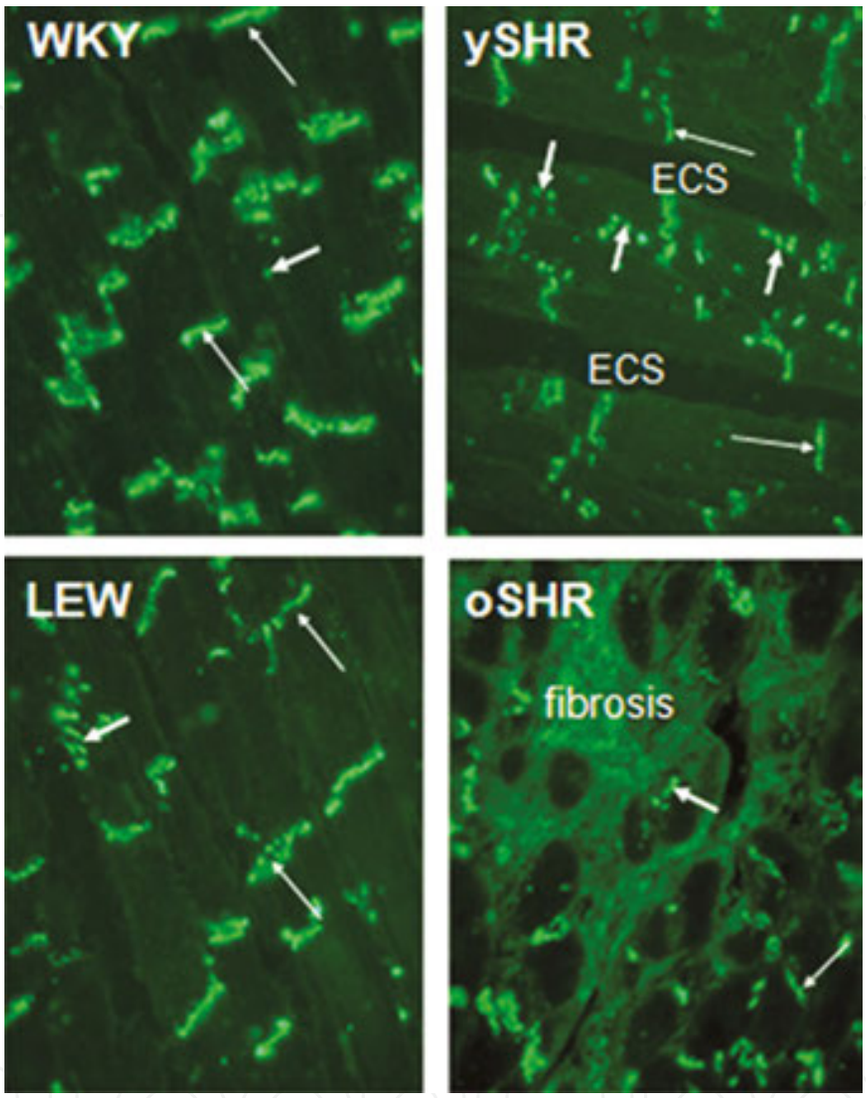

Figure 4. Representative images of myocardial Cx43-immunofluorescence in the left ventricles of young (WKY) and old (LEW) normotensive rats as well as young (ySHR) and old (oSHR) spontaneously hypertensive rat hearts. Note: conventional topology of Cx43-positive gap junctions at the intercalated disks (thin arrows) and enhanced expression of Cx43 on lateral surfaces (short arrows) of the cardiomyocytes in young rats at compensate stage of hypertrophy and pronounced mislocalization of Cx43 due to fibrosis in old rats at decompensate stage of hypertrophy. ESC, extracellular space. Magnification, objective ×40.

3. The role of myocardial Cx43 in protecting from malignant arrhythmias

Therapy to prevent death due to arrhythmias involves invasive procedures, that is, implantable cardioverter defibrillator to protect from sudden cardiac death when VF occurs, catheter

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ablation of arrhythmogenic loci, and resynchronization devices for supporting myocardial synchronization and thereby reducing arrhythmia risk. Blockade of ion current by antiarrhythmic drugs is often ineffective and may even cause proarrhythmia that can increase mortality. As a result, the effort to develop new antiarrhythmic drugs directed at specific ion channels has decreased dramatically. However, mentioned invasive treatment did not prevent the occurrence and/or recurrence of life-threatening arrhythmias. Moreover, these interventions decrease the quality of life and can also be accompanied by various complications. Therefore, novel approach fighting arrhythmia-related sudden cardiac death and stroke is warranted. Considering the crucial role of intercellular coupling and communication to ensure synchronized myocardial contraction, it seems relevant to suggest the implication of these factors in the prevention of arrhythmias.

The novel approach is based on a prevention or attenuation of the development of arrhythmogenic substrates in relation to Cx43 channel function to reduce a risk of arrhythmia occurrence [25, 26]. Taking into consideration, events involved in the development of malignant arrhythmias include oxidative/nitrosative stress, myocardial hypertrophy and/or fibrosis, Cx43 remodeling, and conduction disturbances; the action of antiarrhythmic compounds is expected to include one or more steps in cascade of events [25]. Consistent with it, the "upstream" drug therapy is of great interest aiming to prevent or eliminate arrhythmogenic substrates and triggers. Lipid-lowering drugs, statins, and some antihypertensive drugs are known to exhibit antiarrhythmic effects likely due to the attenuation of myocardial remodeling (hypertrophy, fibrosis) that affects intercellular coupling mediated by Cx43 channels. In addition, these pharmacological compounds exert antioxidant and anti-inflammatory efficacy. All these actions seem to preserve the adequate myocardial Cx43 levels and topology. Direct and/or indirect salutary modulation of Cx43 channel function may confer protection from malignant arrhythmias. Despite the increasing number of experimental studies supporting this idea, there are still many questions to be answered by further research. The topic is challenging to address in experimental as well as in clinical settings or when considering the development of new antiarrhythmic drugs. This article focuses on the benefit of non-pharmacological compounds in spontaneously hypertensive rats, a rodent model mimicking human essential hypertension. It demonstrates novel pleiotropic effects of melatonin and new mechanisms of omega-3 fatty acids and antioxidant-rich red palm oil (RPO) actions that are associated with the modulation of myocardial Cx43.

3.1. Antihypertensive and antiarrhytmic effects of melatonin

Melatonin can reduce blood pressure through the (1) direct effect on hypothalamus, (2) by its antioxidant properties that lower blood pressure, and (3) by decreasing the amount of catecholamines [27]. Hypotensive effect of melatonin could also be mediated by its direct effect on blood vessels or by decreasing serotonin production that is crucial in the inhibition of sympathetic and stimulation of parasympathetic system [28]. Melatonin could affect changes in blood pressure also through its specific melatonin receptors localized in the peripheral vessels or in parts of central nervous system that directly participate in the control of blood pressure [29].

It appears that melatonin in addition to its circadian rhythm regulation exerts a hypotensive effect, and it can modulate cellular redox state and improve the function of the cardiovascular system in pathological conditions. Elderly population has reduced circulating melatonin levels [30, 31]. Decrease of melatonin was also registered in patients suffering from primary hypertension compared with normotensive individuals [32]. Increased melatonin concentration in elderly patients suffering from hypertension may thus be of crucial therapeutic importance. Patients suffering from coronary heart disease, the most typical complication of chronic hypertension, exhibited over fivefold lower level of serum melatonin at night compared with the control group [33]. Likewise, patients demonstrating a "non-dipping profile" of nocturnal arterial pressure exhibited decreased nocturnal melatonin secretion compared with the patients showing a "dipping profile" [34, 35]. The study of Kedziora-Kornatowska et al. [36] confirms the benefit of melatonin supplementation on parameters of oxidative stress in elderly patients suffering from primary hypertension and suggests that melatonin supplementation can be considered as a supporting therapy in the treatment of hypertension. In "non-dippers," a significant hypotensive effect was observed [37]. Melatonin is effective in lowering blood pressure in essential hypertensive patients [38] and in patients with nocturnal hypertension [39]. Antihypertensive effect of exogenous melatonin is reported in numerous experimental studies [29, 39–43].

In addition to antihypertensive effects of melatonin, in vitro and in vivo experimental studies demonstrate its acute antiarrhythmic effects [44–46]. Melatonin appears to be effective even in physiological concentrations. On the other hand, arrhythmias score was significantly higher after coronary artery ligation in pinealectomized rats compared with controls [47, 48]. In almost all of the above mentioned studies, melatonin-induced cardioprotection and antiarrhythmic effects are attributed to its free radical-scavenging potential [44, 49, 50]. Of note, melatonin has several features that are of clinical interest. It has low toxicity, crosses all types of biological barriers (i.e., blood-/brain barrier and placenta), and it can easily enter into all cell compartments including mitochondrias, producers of free radicals [50].

3.2. Antihypertensive and antiarrhythmic effects of omega-3 PUFA

Numerous studies report salutary effects of omega-3 PUFA, that is, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on CVD risk factors [51]. These effects include (1) lowering of serum triglycerides via reduction in hepatic triglycerides production, (2) lowering blood pressure via improved endothelial cell function, (3) decreasing platelet aggregation via reduction in prothrombotic prostanoids, (4) decreasing inflammation via reduction in four-series leukotriene production, and (5) protection from arrhythmia via modulation of electrophysiological properties of cardiac myocytes. Beneficial effects of omega-3 PUFA on CVD risk factors in children including regulation of blood pressure, during childhood, and adolescence are recently reviewed [52]. Systematic meta-analysis suggests that high doses of omega-3 PUFA (~3 g/day) produce a small but significant decrease in systolic blood pressure in older and hypertensive subjects [53].

Observational and interventional studies indicate that dietary omega-3 PUFA may be effective in preventing cardiac arrhythmias and sudden cardiac death. The strongest evidence suggest-

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ing an antiarrhythmic effect of omega-3 PUFA, resulting in significant reduction of sudden cardiac death, provides large GISSI-Prevenzione trial [54]. Antiarrhythmic actions observed in both clinical and experimental conditions are mostly associated with myocardial infarction or post-infarction-related malignant arrhythmias. Some studies, in particular clinical trials, do not clearly demonstrate the antiarrhythmic effects of omega-3 PUFA [55–57]. To explain the discrepancy of the results, it is suggested that the effectiveness of omega-3 PUFA treatment might depend on the mechanism of cardiac arrhythmia, dose, and the route of omega-3 PUFA administration [58]. Moreover, the efficacy of omega-3 PUFA supplementation in clinical trials should be adjusted to initial basal levels of omega-3 PUFA as well as medical treatment regimen of patients. Multiple mechanisms of cardioprotective and antiarrhythmic effects of omega-3 PUFA are suggested, including ion channel function modulation and prevention of pressure overload-related cardiac remodeling [59].

3.3. Cardioprotective and antiarrhythmic effects of red palm oil

The link between dietary fats and cardiovascular diseases has initiated a growing interest in a dietary red palm oil research. RPO is obtained from the orange-red mesocarp of the fruit of a tropical plant known as oil palm *(Elaeis guineensis)* [60]. Besides unsaturated and saturated fatty acids, it contains high concentration of antioxidants such as vitamin A (carotenes), pro-vitamin E—namely tocotrienols, tocopherols, coenzyme Q10, and lycopene [61–63]. In spite of its high level of saturated fatty acid content, RPO intake does not promote vascular disease. On the contrary, the benefits of RPO on health include reduction in the risk of arterial thrombosis and/or atherosclerosis, platelet aggregation, reduction in blood pressure [61], inhibition of endogenous cholesterol biosynthesis, and a reduction in oxidative stress [62]. Oxidative stress and the severity or progression of disease have stimulated further interest in the potential role of RPO (a cocktail of natural antioxidants) to improve redox status. Experimental studies suggest that the cardioprotective effects of RPO may not only be due to the high antioxidant content but could also be mediated by the ability of RPO to modulate signaling events during ischemia and reperfusion [63, 64]. The cardioprotective effects of the tocotrienol-rich fraction have been attributed to the effect of tocotrienol to modulate the Akt signaling, thus generating a survival signal during reperfusion [62]. Beneficial effects of RPO are partially mediated via the phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt) signaling pathway [64]. These findings strongly suggest that PI3K-Akt pathway may play an important role in the RPO-induced cardioprotection. However, this evidence is circumstantial since PI3K has several downstream targets other than Akt. Specific inhibition of Akt will allow to elucidate the importance of Akt on post-ischemic functional recovery in RPO-supplemented animals. RPO supplementation is associated with an increased dual phosphorylation of Akt on Ser473 and Thr308 residues indicating that the optimal activation of Akt requires phosphorylation on both Ser473 and Thr308 residues [65, 66]. Recent experimental studies demonstrate that RPO supplementation offers protection against ischemia/reperfusion injury by improved cardiac output recovery. Evidence strongly suggests that mitogen-activated protein kinases (MAPKs), NO-cGMP, and pro-survival PI3K-Akt signaling pathway may be involved in [63, 64, 66–68]. Further studies are needed to explore novel cellular and molecular mechanisms that might be involved in RPO-related cardioprotection. Data about antiarrhythmic potential of RPO are

rare. According to the recent study [8], increased susceptibility of hyperthyroid rats to malignant arrhythmias is partially ameliorated by supplementation with red palm oil and related mainly to the upregulation of Cx43 and protein kinase C (PKC)ε.

4. Modulation of myocardial Cx43 expression by melatonin

In addition to antiarrhythmic effects of acute administration of melatonin in the setting of ischemia/reperfusion, its antiarrhythmic efficacy has been recently demonstrated in spontaneously hypertensive rats after long-term administration [43]. Several studies showed that compared to normotensive rats, spontaneously hypertensive rats are much prone to develop VF [25]. Consistent with it, the threshold to electrically inducible VF is significantly lower in hypertensive versus normotensive rats but it is increased in response to melatonin treatment. This antiarrhythmic effect of melatonin is associated with the enhancement of myocardial Cx43 gene expression (**Figure 5A**) as well as the total levels of Cx43 protein and its functional phosphorylated forms in hypertensive and to lesser extent in normotensive Wistar rat hearts (**Figure 6**, left panel). Increase of Cx43 phosphorylation could be in part attributed to the increase of PKCε isoform resulting from melatonin treatment. Moreover, Cx43 immunofluorescence labeling and quantitative image analysis reveal an attenuation of abnormal Cx43 distribution and enhanced myocardial Cx43-positive signal in hypertensive rats treated with melatonin. These findings strongly indicate that melatonin may modulate Cx43 expression and distribution in the remodeled heart of hypertensive rats.

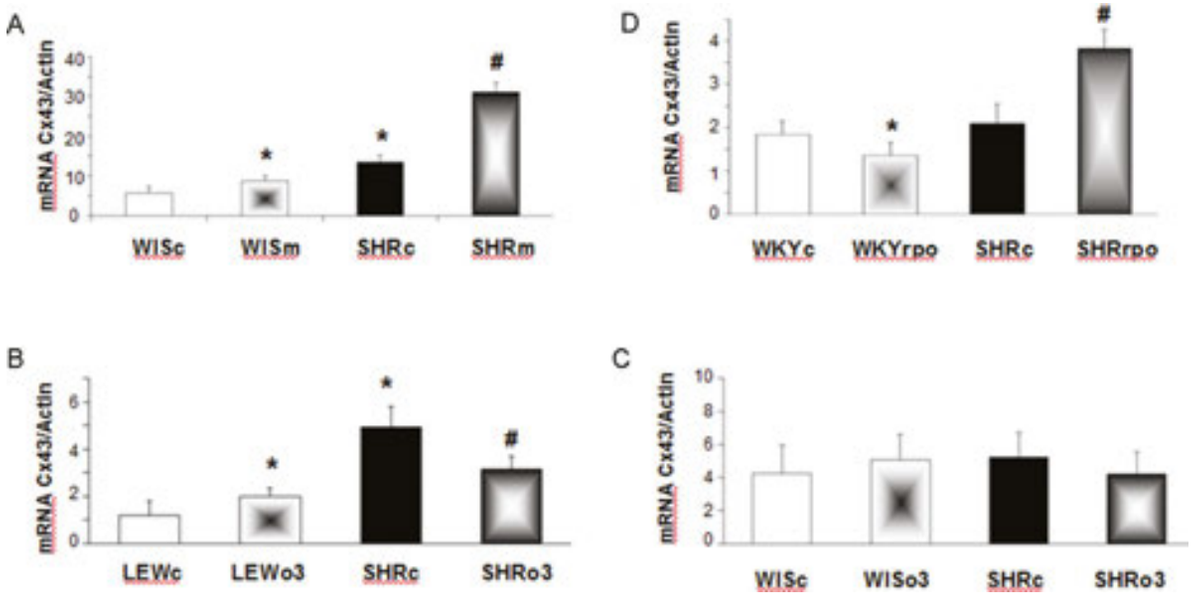

Figure 5. Cx43 mRNA expression normalized to actin in the left ventricles of young (**A**) and old (**B**) untreated and omega-3-treated young SHR and normotensive rat hearts. WKYc, untreated WKY rats; WKYo3, WKY rats treated with omega-3; LEWc, untreated Lewis rats; LEWo3, Lewis rats treated with omega-3; SHRc, untreated SHR; SHRo3, SHR treated with omega-3 PUFA.Results are mean ± SD of eight hearts,significant difference ($P < 0.05$) * from WKYc (WISc, LEWc resp.),and # from SHRc.* Modified with permission from [82]; [43]; [78].

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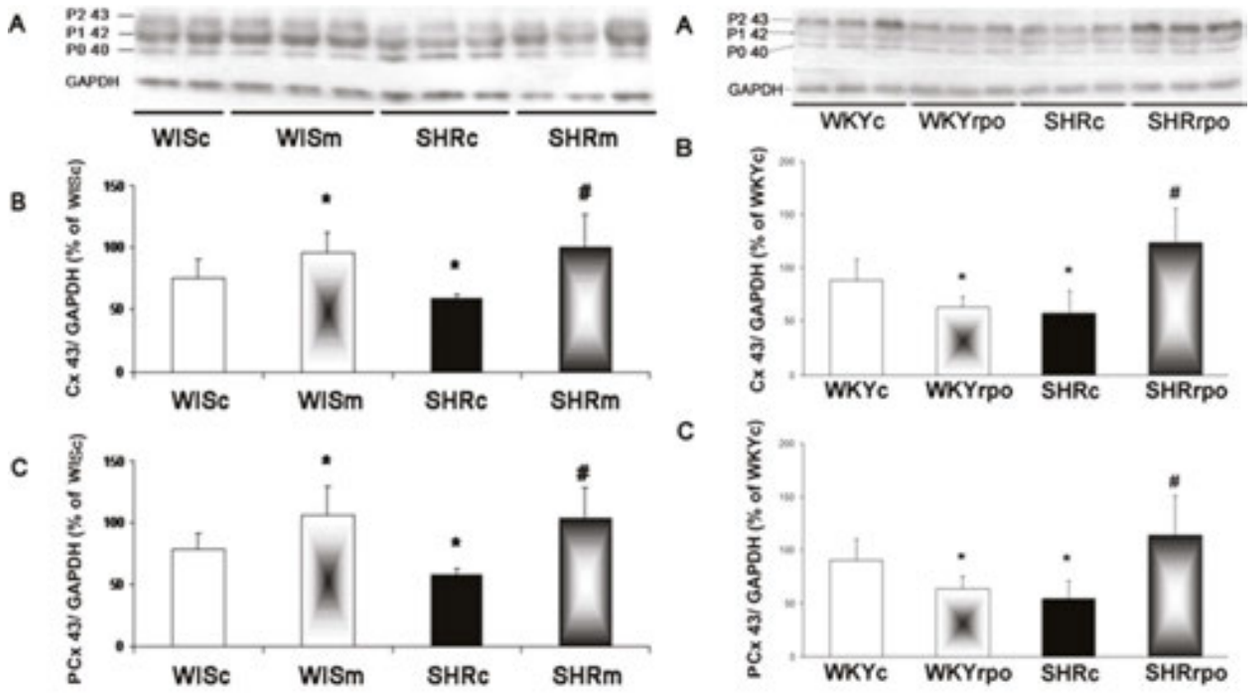

Figure 6. Representative immunoblot (**A**) showing two phosphorylated and one non-phosphorylated form of Cx43 and densitometric quantification of total Cx43 expression (**B**) and its functional phosphorylated forms (**C**) normalized to GAPDH in the left ventricles of untreated and melatonin-treated (left panel), and red palm oil-treated (right panel) hypertensive (SHR) and normotensive (WIS or WKY) rat hearts. Significant difference ($P < 0.05$) * from WKYc (WISc resp.), and # from SHRc. *Modified with permission from [82]; [43].

However, molecular mechanisms of melatonin effects on myocardial Cx43 are not elucidated yet. It has been reported that melatonin upregulates Cx43 (mRNA and protein) and enhances cell-to-cell coupling in human myometrial smooth muscle cells via MT_2 receptor and protein kinase C-dependent manner [69]. It is proposed that melatonin activates phospholipase C followed by the generation of inositol triphosphate and diacylglycerol. The latter activates PKC, which can affect transcription factors c-fos and c-jun that are important in the regulation of Cx43 expression in myometrial cells [70]. Further studies are needed to explore whether this pathway might be involved in the upregulation of Cx43 in heart muscle as well. The cardioprotective and antiarrhythmic effects of acute melatonin treatment in condition of Ischemia/reperfusion were attributed mainly to its antioxidant and free radical-scavenging activity. It is most likely that these cardioprotective actions of melatonin are involved in the condition of oxidative stress induced by hypertension. Consequently, it might result in the preservation of myocardial Cx43 proteins and protection from its downregulation. Melatonin may be classified as a naturally occurring, mitochondrial-targeted antioxidant [71]. This fact is important when considering the most recent studies [26, 72] showing that arrhythmias could be prevented by mitochondria-targeted antioxidants rather than by general antioxidants. It also seems possible that melatonin could protect myocardial Cx43 via inhibiting the activity of cyclooxygenase 2 and inducible NO synthase caused by chronic inflammation [73]. Hypo-

thetical mechanisms of the modulation of cardiac Cx43 channels and protection from malignant arrhythmias by melatonin are depicted on **Figure 7**.

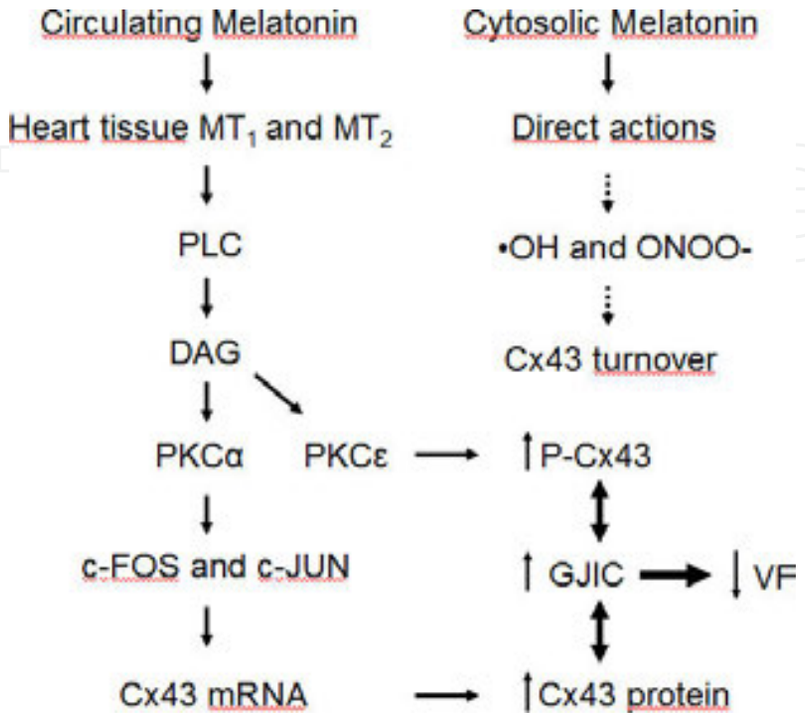

Figure 7. Proposed mechanisms of melatonin action on myocardial Cx43 expression and distribution in spontaneusly hypertensive rat heart. Circulating melatonin via its receptors, MT_1 and MT_2, activates phospholipase C (PLC) followed by the production of diacylglycerol (DAG), which activates protein kinase C (PKC). PKCε by phosphorylation of Cx43 can modulate the channel's function as well as its myocardial distribution and subsequently gap-junctional intercellular communication (GJIC). Activation of PKC*α* also affects transcription factors c-FOS and cJUN, which bind to conserved activator protein-1 in the promoter region of Cx43 and hence can increase Cx43 expression. In addition, melatonin exhibits receptor-independent actions due to its ability to scavenge free radicals. Free radicals enhance the degradation of Cx43 and melatonin can attenuate this process and preserve myocardial Cx43 levels. Altogether, the protection of functional Cx43 by melatonin can affect GJIC and improve electrical stability resulting in the decrease of inducible VF.

5. Modulation of myocardial Cx43 expression by omega-3 PUFA

A direct renin inhibitor, aliskiren, and dietary omega-3 PUFA attenuate electrical remodeling in renin-angiotensin transgenic rats (another model mimicking human hypertension) most likely due to the restoration of normal topology of Cx43 [74]. Both treatments also reduce the QRS and QT interval suggesting an improvement in conduction that could be attributed to reduced fibrosis and the elimination of lateral distribution of Cx43. Consequently, it results in the decline of tachyarrhythmia induction. In addition, aliskiren and omega-3 PUFA prevent hypertension-related inflammation that is generally known to downregulate Cx43 [75].

Antiarrhythmic effects of both omega-3 PUFA and atorvastatin (a hypolipidemic drug with anti-inflammatory and antioxidant properties) are demonstrated in hereditary hypertrigly-

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ceridemic rats with elevated blood pressure [76]. The decrease of VF inducibility is associated with the suppression of hyper-phosphorylation of Cx43 and the restoration of its normal myocardial topology. Electron microscopy examination reveals that both omega-3 PUFA and atorvastatin improve the structural integrity of mitochondria, plasma membrane, and intercellular junctions when compared to untreated diseased rats. This membrane protective effect may be partially explained by changes in membrane composition [77], that is, increased incorporation of omega-3 PUFA and decreased cholesterol levels due to treatments. It would be interesting to know whether atorvastatin affects properties of ion and Cx43 channels as well as well as myocardial conduction, (likewise omega-3 PUFA), to understand better its antiarrhythmic effect in relation to myocardial Cx43 alterations.

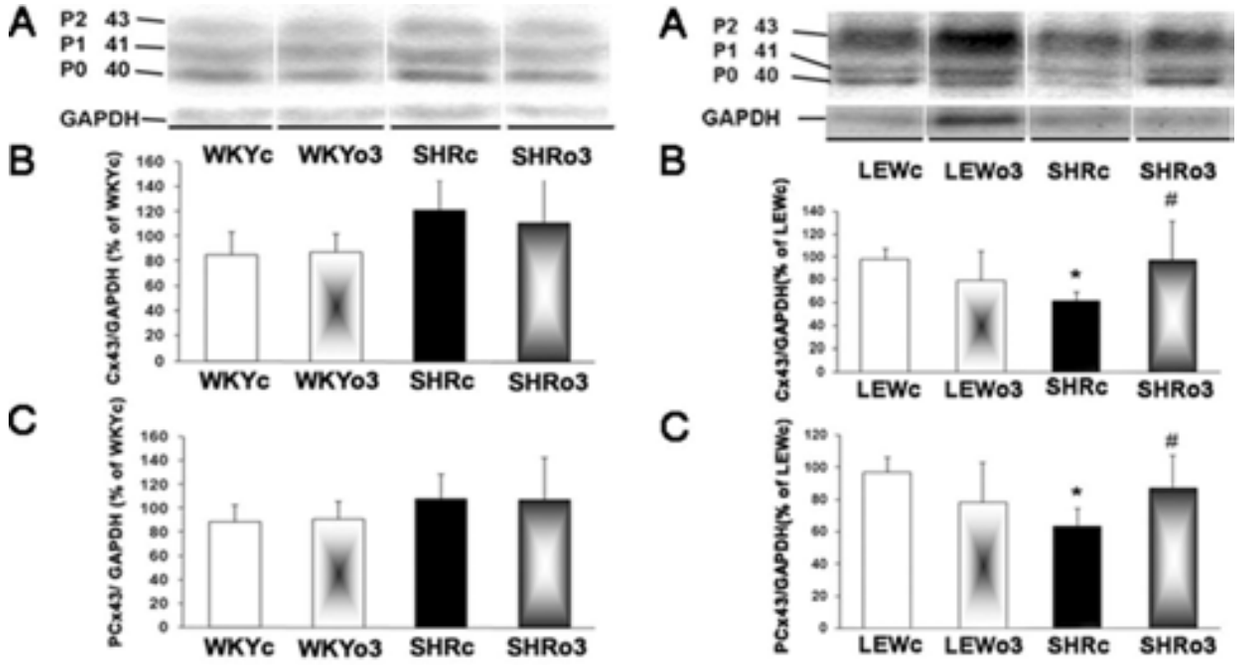

Figure 8. Representative immunoblot (**A**) showing three forms of Cx43 and densitometric quantification of total Cx43 expression (**B**) and its functional phosphorylated forms (**C**), normalized to GAPDH in the left ventricles of untreated and omega-3-treated young SHR and WKY rat hearts (left panel) as well as in the left ventricles of untreated and omega-3-treated old SHR and Lewis rat hearts (right panel). Results are mean ± SD of eight hearts, Significant difference ($P < 0.05$) * from LEWc, and # from SHRc. *Modified with permission from [78].

Protection from VF due to intake of omega-3 PUFA (i.e., DHA and EPA) and the implication of myocardial Cx43 are demonstrated in another study [78] using young (compensated stage of hypertrophy) and old spontaneously hypertensive rats (early decompensated stage of hypertrophy). Findings show that omega-3 PUFA intake normalizes myocardial Cx43 mRNA levels in old rats (**Figure 5C**, **D**) and Cx43 protein expression as well as its functional phosphorylated status in both young and old hypertensive animals (**Figure 8**). Enhanced Cx43 phosphorylation might be in part attributed to PKCε that is upregulated by omega-3 PUFA. The treatment significantly eliminates abnormal Cx43 distribution (**Figure 9**), diminishes the

internalization of gap junctions, and improves ultrastructure (integrity) of the mitochondria in cardiomyocytes of hypertensive rats. These findings clearly indicate that the modulation of Cx43 channel function and myocardial cell-to-cell coupling by omega-3 PUFA might be possible.

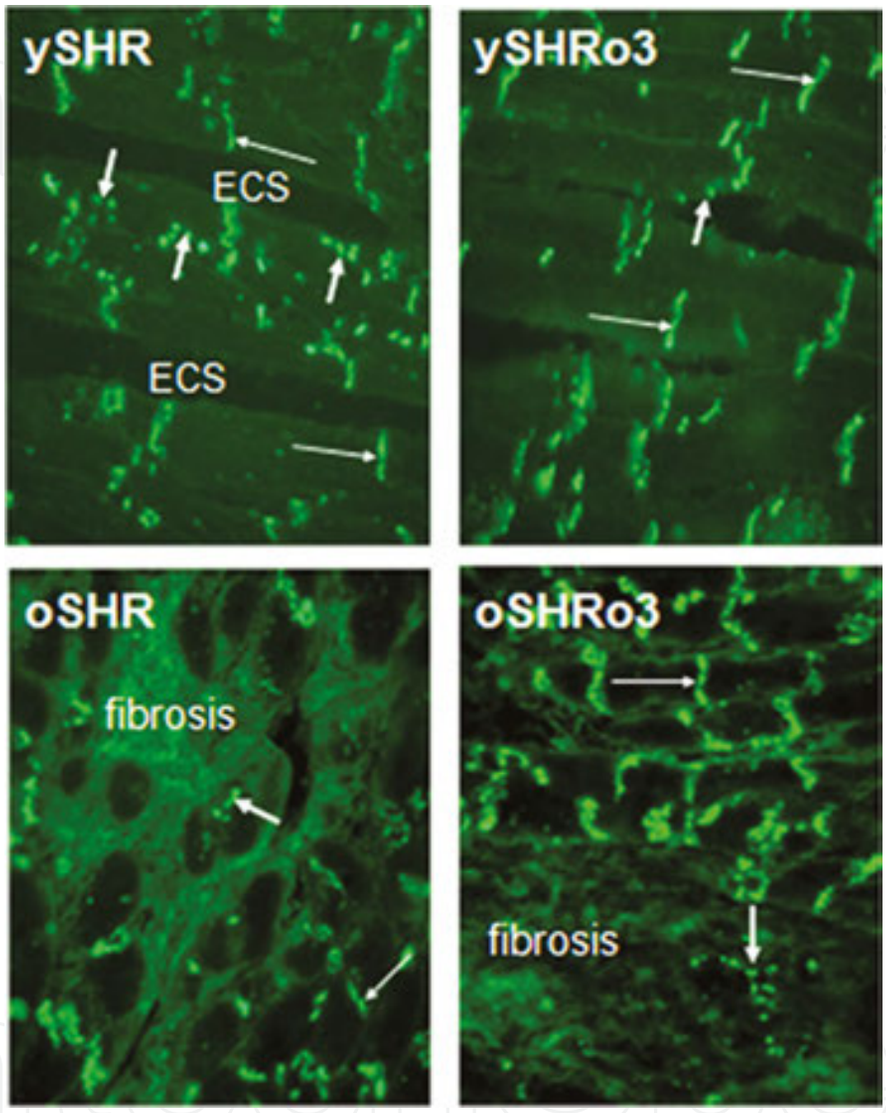

Figure 9. Representative images of myocardial Cx43-immunofluorescence in the left ventricles of untreated young (ySHR) and old (oSHR) and omega-3 fatty acid-treated young (ySHRo3) and old (oSHRo3) spontaneously hypertensive rat hearts. Note: prevalent normal Cx43 distribution at the intercalated disks (thin arrows) and apparent attenuation of disordered Cx43 distribution (short arrows) as well as reduced fibrosis due to supplementation with omega-3 fatty acids. ESC, extracellular space. Magnification, objective ×40.

Nevertheless, the question arises as to how omega-3 affects myocardial Cx43 expression and its phosphorylation. It is known that omega-3 PUFA are ligands for the nuclear transcription factor, peroxisome proliferator-activated receptor (PPAR). It is also known that omega-3 PUFA might regulate numerous gene expressions and consequently intracellular pathways involved in protein expression and phosphorylation [79, 80]. The discovery of omega-3 PUFA signaling

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pathways linked with Cx43 modulation may reveal new candidates for antiarrhythmic drug development. Antiarrhythmic properties of omega-3 PUFA may also include the modulation of membrane ion current densities and intracellular Ca^{2+} handling [81]. Available data suggest that possible mechanisms of omega-3 PUFA (shown in **Figure 10**) could prevent downregulation and mislocalization of cardiac Cx43. Consequently, it could lead to suppression in the incidence of life-threatening arrhythmias.

Figure 10. Diagram for the proposed mechanisms of omega-3 PUFA that could prevent downregulation and mislocalization of cardiac Cx43. Consequently, it could lead to a reduction in the incidence of life-threatening arrhythmias.

6. Modulation of myocardial Cx43 by red palm oil

It has been, for the first time, demonstrated that there is upregulation of myocardial Cx43 and suppression of PKCε activation in response to RPO supplementation of male, adult spontaneously hypertensive rats [82]. In this study, Cx43-mRNA (**Figure 5D**), total Cx43 proteins, and its phosphorylated forms are increased. Moreover, disordered localization of Cx43 is attenuated in the left ventricle of RPO-fed hypertensive rats compared with untreated rats. These alterations are associated with the suppression of early post-ischemic-reperfusion-related VT and electrically inducible VF. Moreover, it is linked with the improvement of functional recovery of the heart during post-ischemic reperfusion. However, the treatment dose of RPO (200 mg/day for 5 weeks) causes downregulation of myocardial Cx43 in normotensive age-matched rats. It results in poor arrhythmia protection, suggesting overdosing of

RPO in healthy rats. Findings indicate that hypertensive rats benefit from RPO intake, particularly because of its apparent antiarrhythmic effects. This protection can be, in part, attributed to the upregulation of myocardial Cx43 but not with PKCε activation. In addition, RPO supplementation reduced blood pressure in hypertensive rats and blood glucose in both hypertensive and normotensive rats. Taken together, the results indicate that hypertensive rats benefit from RPO supplementation, particularly due to its apparent antiarrhythmic and post-ischemic-reperfusion-related cardioprotective effects that can be, in part, explained by the upregulation of myocardial Cx43. This view is supported by findings that the downregulation of Cx43 in response to RPO intake of healthy normotensive rats is associated with poor antiarrhythmic effect.

7. Conclusions and perspectives

Data included in this comprehensive article suggest that the attenuation of hypertension-induced abnormal and/or restoration of normal myocardial expression and distribution of Cx43 as well as enhancement of its functional phosphorylated forms along with positive modulation of PKC signaling by melatonin, omega-3 fatty acids, and red palm oil may be crucial in their antiarrhythmic mechanisms. Despite "optimal" therapy of patients suffering from hypertension, there is still urgent need for preventing severe rhythm disorders. In view of the many missed potential targets for preventing adverse myocardial remodeling, it appears that the beneficial modulation of Cx43 by melatonin, omega-3 fatty acids, and red palm oil might be a useful approach in current therapy. Our findings support the prophylactic use of these non-pharmacological compounds to minimize cardiovascular risk and sudden arrhythmic death. A novel approach is needed since despite a plethora of available treatment options, a substantial portion of the hypertensive population has uncontrolled blood pressure. Further studies should elucidate more in detail mechanisms of myocardial Cx43 modulation in the context of electrical properties of the heart in response to treatment of hypertension

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Author details

Tamara Egan Benova, Barbara Szeiffova Bacova, Csilla Viczenczova, Miroslav Barancik and Narcis Tribulova*

*Address all correspondence to: narcisa.tribulova@savba.sk

Institute for Heart Research, SAS, Bratislava, Slovakia

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